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Sorbitol as the Primary Carbon Source for the Growth of Embryogenic Callus of Maize[1]

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The effects of various carbon sources on initiation and maintenance of embryogenic callus of maize (*Zea mays* L.) and on the regeneration of plants from embryogenic callus were studied. Growth of embryogenic callus tissue on media containing sucrose was typified by the subsequent growth of both embryogenic (regenerable) and nonembryogenic (nonregenerable) callus. Growth of embryogenic callus on sorbitol was unique among the carbon sources tested in that sorbitol supported the subsequent growth of only embryogenic callus. Further experiments demonstrated that embryogenic callus grown on sorbitol had a greater regenerative capacity (more plants produced per gram fresh weight of callus) than callus grown on sucrose. Sorbitol dehydrogenase was detected in embryogenic callus of maize at a specific activity roughly equivalent to that found in zygotic embryos of developing seeds. Nonembryogenic callus did not contain significant levels of sorbitol dehydrogenase activity.

Because of their economic importance, cereal species are attractive targets for crop improvement utilizing recently developed techniques of molecular biology. A significant step in improving cereal transformation techniques has been the recognition of embryogenic callus culture systems as a viable means of growing regenerable cereal tissue cultures (Vasil, 1987). These cultures have been successfully used as a source of regenerable protoplasts (Fujimura et al., 1985; Srinivasan and Vasil, 1986; Prioli and Sondahl, 1989; Shillito et al., 1989) and have also been used in transformation experiments yielding transgenic plants (Rhodes et al., 1988; Gordon-Kamm et al., 1990). Yet, in maize (*Zea mays* L.) and many other cereals, embryogenic cultures are often difficult to obtain and require some degree of skill to identify and maintain. For example, friable embryogenic cultures used to procure embryogenic suspensions from which regenerable protoplasts can be obtained are apparently difficult to distinguish from nonembryogenic (nonregenerable) callus (Prioli and Sondahl, 1989; Shillito et al., 1989).

A number of factors have been examined in an effort to improve growth of embryogenic callus of the cereals (Duncan et al., 1985; Imbrie-Milligan and Hodges, 1986; Close and Ludeman, 1987). However, one area of investigation that has received little attention is carbohydrate utilization in vitro. Straus and LaRue (1954) first investigated the effects of various carbohydrates on the growth of maize endosperm cultures and determined that Suc was most effective in supporting growth of maize endosperm cultures. Since this early study, very little has been reported relative to alternative carbon sources in the culture of monocots, with the distinct exception of several reports on carbon utilization in cultures of sugarcane. From studies utilizing a wide range of carbon sources, Nickell and Maretzki (1970) and Maretzki et al. (1974) concluded that, based on culture fresh weight gain, Suc was the carbon source of choice for sugarcane.

Most studies on in vitro carbohydrate utilization have drawn conclusions regarding the carbon source for optimal growth. In nearly all cases, Suc, Glc, and/or Fru support the greatest gains in culture weight, often with little difference among them. Thus, it is generally concluded that Suc is the best carbon source to use because it supports nearly optimal rates of growth and it is relatively inexpensive to use.

By comparison, minimal attention has been given to associated culture morphology and to effects on morphogenesis in the cultures grown on varying carbon sources. A few reports have noted that the carbohydrate source can have an effect on the degree and type of differentiation, and thus on the morphology of the cultures (Verma and Dougall, 1977; Alsop et al., 1981; Kochba et al., 1982; Strickland et al., 1987). Various carbon sources other than Suc have been used to enhance androgenesis (Babbar and Gupta, 1986; Batty and Dunwell, 1989; Last and Brettel, 1990) or somatic embryogenesis (Kochba et al., 1982; Strickland et al., 1987; Nadel et al., 1989). Although many aspects of carbon metabolism are well documented, it is apparent that our knowledge of carbon metabolism and its relationship to growth and morphology is limited.

The observations reported here primarily concern the growth of embryogenic callus of maize on media containing sorbitol, a six-carbon sugar alcohol, as the primary carbon source. Particular attention is directed at the influence of sorbitol on the morphology of maize cultures and on the subsequent influence on the regenerative capacity of the callus.

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Abbreviations: dpp, days postpollination; MS, inorganic salt formulation of Murashige and Skoog (1962); N6, inorganic salt formulation of Chu et al. (1975); SDH, sorbitol dehydrogenase.

MATERIALS AND METHODS

Plant Material and Culture Initiation

Maize (*Zea mays* L.) inbred line H99 was grown in the field in Salt Lake City, UT, and self pollinated. Immature embryos approximately 1.5 to 2.5 mm in length taken from kernels 10 to 15 dpp were aseptically excised according to the procedure described by Duncan et al. (1985). Excised embryos were cultured in darkness at 22°C with the embryo axis in contact with the media. The culture medium was essentially medium B as described by Duncan et al. (1985) except that the medium contained 87.5 mM Suc in place of 60 mM Suc. After approximately 4 weeks, embryogenic callus was visually selected and subcultured to fresh media.

For comparisons of carbohydrate effects on culture initiation, immature embryos were cultured as described above with the exception that 87.5 mM Suc was replaced with 87.5 mM Fru, Glc, or sorbitol. Media were coded to eliminate observer bias and, after 4 weeks, embryos forming embryogenic callus were visually evaluated by three independent observers. Individual embryos were scored for the amount of embryogenic callus formed.

Culture Maintenance

Embryogenic callus was maintained in darkness at 22°C on the same Suc-containing media as described above by visual selection of sectors of embryogenic callus and subculture to fresh media. A subculture interval of 4 weeks was used, and embryogenic callus used in these experiments was maintained on Suc-containing medium for a minimum of 2 months following initiation to ensure uniform material for experiments. Callus of varying ages was used between replications and experiments, but in experiments where head-to-head comparisons are shown, embryogenic callus of the same age was used within replications of each experiment. No embryogenic callus that had been in culture for more than 8 months was used in any experiment.

Embryogenic callus growing on Suc was transferred to media containing one of the following carbohydrates: Xyl, Rib, Glc, Fru, Gal, maltose, lactose, cellobiose, melibiose, raffinose, sorbitol, or *myo*-inositol at 2% (w/v) concentrations. Cultures were incubated for 1 month on the new carbohydrate. Then for each of five plates per treatment, five pieces of embryogenic callus derived from these cultures totaling approximately 100 mg each were transferred to fresh media containing the same carbohydrate. Fresh weight gains were monitored weekly for 4 weeks, and the experiment was repeated at least twice for each carbohydrate.

Suc and sorbitol were further compared by monitoring fresh weight gains of callus grown on 87.5, 175.0, or 262.5 mM Suc or 87.5. 175.0, 262.5, or 350.0 mM sorbitol. In addition, the effects of Suc (87.5 mM) and sorbitol (87.5 mM) on callus growth rates and culture morphologies were compared when the two carbohydrates were used in conjunction with an MS or N6 inorganic salt base.

Plant Regeneration

Plants were regenerated from embryogenic callus by reducing the level of dicamba in the media from 30 to 3 μM and adding 1 μM kinetin. For comparison of carbon source effects, pieces of embryogenic calli derived from calli that had been maintained on Suc or sorbitol for a minimum of three subcultures and that had been in culture for a total of 4 months were sieved through an 800-μm screen to separate calli into small cell aggregates. Care was taken to ensure that only calli that were distinctly embryogenic were weighed, sieved, and transferred to regeneration media. Prior to use in these comparisons, conditions were established for optimum regeneration of plants from embryogenic callus. It was determined that the greatest number of shoots developed per g fresh weight of callus when the callus was mechanically dispersed by forcing it through an 800-μm sieve (Belco, Cellector, Vineland, NJ). Unsieved callus and other sieve sizes produced fewer shoots per g fresh weight of embryogenic callus (data not shown).

Sieved embryogenic callus was weighed and transferred to regeneration media containing either Suc or sorbitol. Two hundred to 300 mg of callus were placed on each of five Petri plates. Cultures were placed in a lighted growth room at 25°C with a 16-h photoperiod provided by Sylvania Cool White fluorescent lamps at a photon density of approximately 70 μmol photons m^{-2} s^{-1}. After 3 and 6 weeks, shoots 1 cm in length or longer were counted on each Petri plate, and comparisons were made based on the number of shoots formed per g fresh weight of the original callus. The experiment was repeated twice, and data shown are the per plate means of the 10 plates from the two replications.

SDH

SDH assays were conducted as described by Doehlert (1987) on dialyzed ammonium sulfate fractions prepared as described below. In a typical preparation, 5 g (fresh weight) of tissue were ground in 50 mL of prechilled buffer solution consisting of 100 mM Tris-HCl (pH 8.5), 1 mM DTT, and 5 mM $MgCl_2$. Homogenization and all subsequent steps were performed at 4°C. The solution was passed through a double layer of Miracloth and centrifuged at 1,000*g* for 10 min. The supernatant was collected and recentrifuged at 20,000*g* for 10 min. The supernatant was made 30% saturated with ammonium sulfate and then centrifuged at 20,000*g* for 10 min. The supernatant was collected, made 70% saturated with ammonium sulfate, and centrifuged at 20,000*g* for 10 min. The precipitate was resuspended in buffer at one-twentieth the original sample volume. This solution was dialyzed overnight against three changes of Tris extraction buffer as described above and used in enzyme assays. Protein determinations were made using the method of Bradford (1976).

Statistical Methods

For all fresh weight gain studies there was a tendency for the variance to increase as the percent increase in fresh weight became larger. Therefore, the percent increase was transformed to the square root of the percent increase prior to application of statistical techniques. Post-hoc orthogonal contrasts were used to determine differences in treatment means. Levene's test (1960) was employed to examine differences in the amount of variation about the mean.

RESULTS

Initiation of Embryogenic Callus

Of the four carbohydrates tested for initiation of embryogenic callus, a higher percentage of embryos formed embryogenic callus on Suc (25%) than on Fru (1%), Glc (10%), or sorbitol (16%). Observations of calli initiated on these carbon sources suggested that embryogenic calli forming on Suc tended to be larger than calli forming on the other carbohydrates, and the extent of embryogenic callus formation visually correlated with the percentage of embryos forming embryogenic callus in each carbohydrate treatment.

Maintenance of Embryogenic Callus

Nine of the 13 carbohydrates tested supported some callus growth (Table I). Rib, melibiose, raffinose, and inositol did not support callus growth. Lactose and Gal supported limited growth of nonembryogenic callus but not embryogenic callus. When subcultured on these two carbohydrates, the callus growth rate gradually declined, the callus necrosed, and after three to five subcultures all growth ceased. Consequently, growth data for these two carbon sources are not given in Table I. Each of the seven remaining carbohydrates supported the growth of some embryogenic callus.

Fresh weight of tissue grown on maltose was significantly greater than that grown on any other carbohydrate tested. Glc-, Fru-, and Suc-containing media supported growth rates not significantly different from each other but lower than media containing maltose, and cellobiose and sorbitol were similar to each other but supported significantly less growth than Glc, Fru, or Suc. Xyl supported the least growth of any of the carbon sources tested except lactose and Gal, which supported limited and unsustained growth.

Glc, Fru, maltose, Suc, and cellobiose all supported both embryogenic and nonembryogenic callus growth. Callus grown on Glc, maltose, and particularly Fru was more nonembryogenic-type callus than was callus grown on Suc. However, if pieces of embryogenic callus were carefully selected at each subculture, cultures grown on these carbon sources would continue to develop enough embryogenic callus to sustain an embryogenic culture.

When embryogenic callus was subcultured onto media containing Xyl, the callus would turn brown rapidly, appear to necrose, and then white, embryogenic callus would appear on the surface of the brown tissue. Upon subculture onto fresh media containing Xyl, the callus would repeat this cycle by again turning brown, becoming necrotic, and then forming very white embryogenic callus on its upper surface. This cycle has been repeated for four culture cycles with no apparent loss of capacity to form embryogenic callus. No attempts have been made to continue beyond four cycles.

Callus growing on sorbitol was almost entirely embryogenic. The callus consisted of white- to cream-colored tissue very similar to what is commonly referred to as "type 1" embryogenic callus in maize (Fig. 1). It differed from type 1 embryogenic callus of maize grown on Suc in that embryoids with a distinct coleoptile and radicle and with bilateral symmetry developed less frequently on sorbitol. Embryoids that did develop on sorbitol were smaller than those that developed on Suc (Figs. 2 and 3). Callus grown on sorbitol seldom developed radially symmetric embryoids typical of "type 2" callus, nor was it as friable as type 2 callus.

Comparisons of fresh weight gains of calli maintained on

Table I. *Inbred H99 callus growth on various carbohydrates*

Embryogenic callus growing on Suc (see "Materials and Methods") was harvested and transferred to media containing the various carbohydrates listed (2% [w/v] concentration). The type of growth (embryogenic versus nonembryogenic) and the fresh weight of the growing tissue at weekly intervals are shown.

Carbohydrate	Embryogenic Callus[a]	Weekly Percent Increase in Fresh Weight[b]			$P < 0.01$[c]
		Week 1	Week 2	Week 3	
Maltose	+	102	315	707	a
Suc	+	87	272	446	b
Glc	+	96	263	441	b
Fru	+	92	253	398	b
Cellobiose	+	65	194	356	c
Sorbitol	+	66	183	317	c
Xyl	+	60	130	244	d
Lactose	−		N/D[d]		
Gal	−		N/D		
Rib	−		No Growth		
Melibiose	−		No Growth		
Raffinose	−		No Growth		
Inositol	−		No Growth		

[a] +, At least some embryogenic callus formed; −, no embryogenic callus formed. [b] Numbers represent the average percent increase in fresh weight gain calculated as follows {[(weight at week X) − (beginning weight)/(beginning weight)] × 100}. [c] Letters signify statistically similar (same letter) or different (different letter) growth rates by orthogonal contrast ($P < 0.01$), comparing carbon sources across all weeks. [d] N/D indicates growth rates not determined.

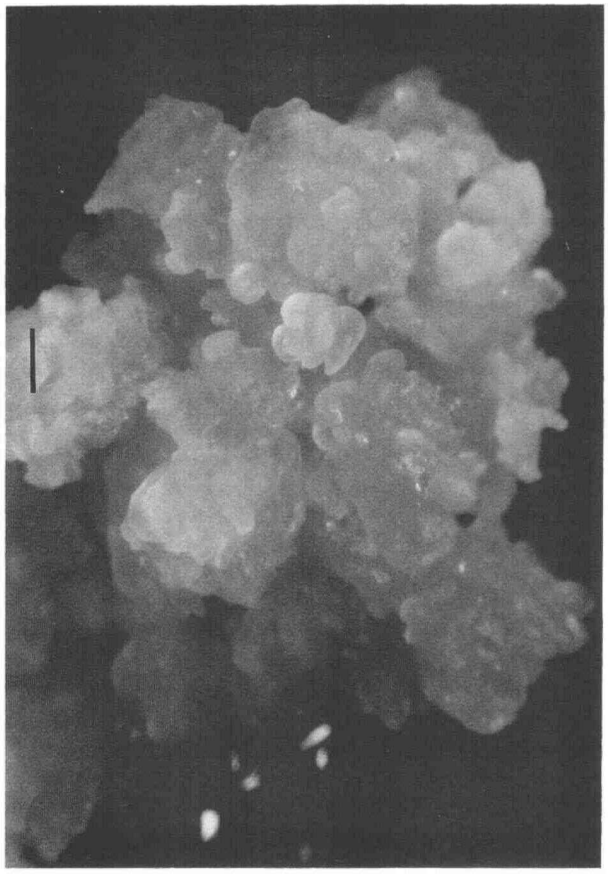

Figure 1. Embryogenic callus of maize grown on 175 mM sorbitol for three subcultures with 30 μM dicamba. Callus is generally white to cream colored and develops bilaterally symmetrical embryoids without well-defined embryo axes. It is similar to "type 1" maize embryogenic callus grown on 87.5 mM Suc with 30 μM dicamba except that bilaterally symmetrical embryoids are not as numerous and embryoids typically do not develop well-defined embryo axes to the extent that they do on Suc. Bar = 1 mm.

Suc (87.5, 175.0, or 262.5 mM) or sorbitol (87.5, 175.0, 262.5, or 350.0 mM) indicated that Suc supported a significantly larger gain in fresh weight than sorbitol regardless of the concentration used (Table II). Although there was no significant difference in fresh weight gain among the three concentrations of Suc tested, changes in culture morphology associated with changes in Suc concentrations were apparent. The lowest concentration of Suc (87.5 mM) was superior to the higher concentrations in terms of the amount of embryogenic callus formed. Callus grown on 262.5 mM Suc formed numerous roots in association with nonembryogenic callus, and it was difficult to select and maintain embryogenic callus on this high concentration of Suc.

The lowest concentration of sorbitol (87.5 mM) promoted significantly better growth than the other sorbitol concentrations, and the highest concentration of sorbitol (350.0 mM) promoted the least growth. The morphology of calli grown on sorbitol did not vary appreciably from one concentration to another, although, at the highest concentration of sorbitol (350 mM), necroses was evident in portions of the callus. At the three lowest sorbitol concentrations, the calli appeared to be entirely embryogenic.

Growth rates were also compared for either MS or N6 inorganic salt-based media, each amended with either Suc or sorbitol (Table III). For calli grown on Suc, the mean fresh weight gains were statistically similar on N6 and MS. However, when replicate cultures were compared, the growth rates varied substantially from one replicate to another when calli were cultured on MS-based media, whereas variations between replicates on N6-based media were much less pronounced. This observation was confirmed statistically by Levene's test (1960), which tests differences of variation about the means of two populations whose means themselves are not statistically different. When an individual piece of callus became entirely nonembryogenic, its growth rate appeared to decline significantly. The number of pieces becoming nonembryogenic varied significantly from plate to plate when the tissue was grown on MS media.

Callus growth patterns and callus morphology were similar for callus grown on sorbitol regardless of the salt base. Differences in the calli, including differences in fresh weight gains, were not obvious, nor was there statistical evidence of differences in either the mean growth rates or variation about the mean.

Plant Regeneration

Gross comparisons of plant regeneration on Suc, Fru, Glc, and sorbitol clearly indicated that plant regeneration from embryogenic callus was superior when callus had been grown on Suc or sorbitol rather than on Fru or Glc (data not shown).

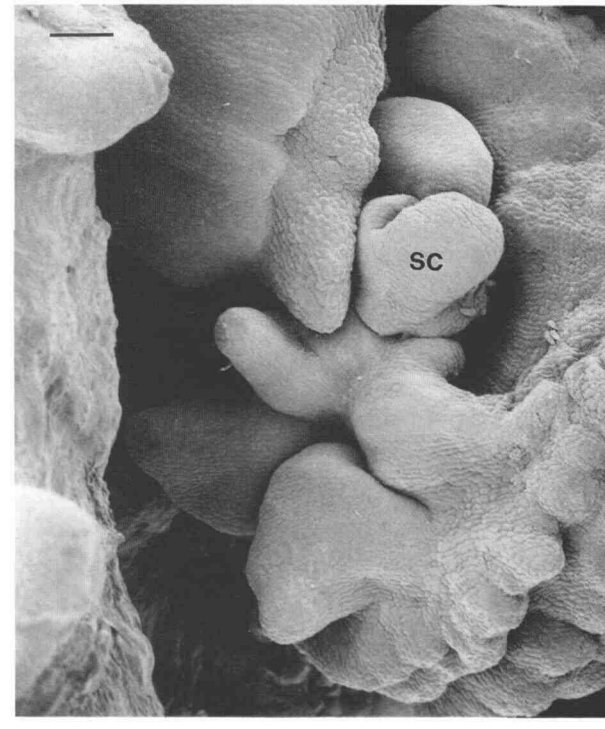

Figure 2. Scanning EM of embryogenic callus grown on 175 mM sorbitol. Note numerous scutellar structures exhibiting early stages of bilateral symmetry but without well-defined embryo axes. Embryoids only occasionally developed well-defined axes. However, when dicamba was reduced or removed, a typical embryo axis would develop and plants would form. Bar = 100 μm; SC, scutellum.

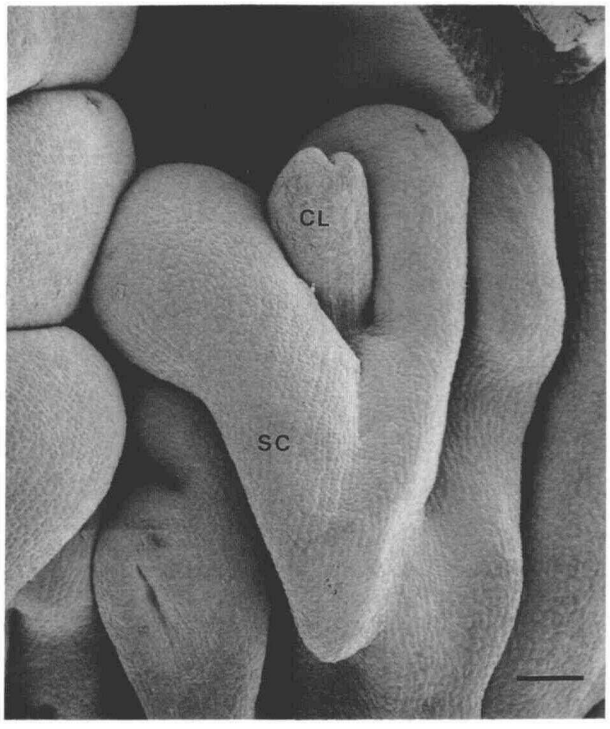

Figure 3. Scanning EM of callus and an embryoid typical of those frequently observed on "type 1" embryogenic callus grown on Suc. Note the well-developed structures typical of a mature zygotic embryo. Bar = 100 μm; CL, coleoptile; SC, scutellum.

The regeneration capacity of H99 callus grown on Suc or sorbitol showed that for callus grown on Suc, there was no significant difference between regeneration on sorbitol or Suc (Table IV). However, for callus grown on sorbitol, regeneration was significantly higher when the regeneration media also contained sorbitol. Regardless of the regeneration media, callus grown on sorbitol formed significantly more shoots per g fresh weight of embryogenic callus than callus grown on Suc.

Table II. *Inbred H99 callus growth on various levels of Suc and sorbitol*

Maize embryogenic callus tissue was transferred to media containing various millimolar concentrations of Suc or sorbitol. Weekly percent increase in fresh weight calculated as in Table I was determined.

Sugar	mM	Weekly Percent Increase in Fresh Weight			P < 0.01[a]
		Week 1	Week 2	Week 3	
Suc	87.5	125	288	482	a
Suc	175.0	125	290	506	a
Suc	262.5	119	243	402	a
Sorbitol	87.5	101	207	286	b
Sorbitol	175.0	73	173	245	c
Sorbitol	262.5	57	141	221	c
Sorbitol	350.0	38	97	150	d

[a] Letters signify statistically similar (same letter) or different (different letter) growth rates by orthogonal contrasts (P < 0.01), comparing carbon sources × concentrations across all weeks.

Table III. *The growth of maize embryogenic callus on MS or N6 basal media containing Suc or sorbitol*

Means and SD values for percent increase in fresh weight of callus of maize grown on different basal media amended with either Suc or sorbitol are shown at weekly intervals for 3 weeks. Note: Even though the means of fresh weight gain did not differ between calli grown on Suc on MS or N6, there was significantly more variation in growth rate within the treatment when the callus was grown on MS as opposed to N6 (Levene's test, P < 0.01).

Basal Media	Carbon Source	Weekly Percent Increase in Fresh Weight[a]			P < 0.01[b]
		Week 1 Mean (SD)	Week 2 Mean (SD)	Week 3 Mean (SD)	
MS	Suc	155 (115)	300 (236)	482 (377)	a
N6	Suc	94 (53)	252 (123)	495 (195)	a
MS	Sorbitol	79 (46)	192 (117)	261 (127)	b
N6	Sorbitol	59 (45)	135 (83)	243 (116)	b

[a] Percent increase in fresh weight calculated as in Table I. [b] Letters signify statistically similar (same letter) or different (different letter) growth rates by orthogonal contrasts (P < 0.01), comparing carbon sources × basal media across all weeks.

SDH

SDH activity was detected in embryogenic callus of maize. However, nonembryogenic callus showed little or no SDH activity. Table V lists the specific activities of SDH in embryogenic callus, nonembryogenic callus, various tissues of 21 dpp developing kernels, and leaf and root tissues from maize seedlings. The specific activity per mg of protein was highest for homogenates prepared from basal endosperm. Kernel bases, middle endosperm, apical endosperm, and embryo tissues all had substantial SDH activities in decreasing order, respectively. Pericarp tissue contained little measurable SDH activity, and the level of SDH present in seedling leaves and roots was below the level of detection in the assay. Homogenates from embryogenic callus also had high specific activity. This activity was comparable with zygotic embryos and with apical endosperm tissue. Homogenates derived from

Table IV. *The effects of Suc and sorbitol on the regeneration of plants from embryogenic callus*

Callus was maintained on media with Suc or sorbitol as the carbon source and transferred to regeneration media containing Suc or sorbitol. Numbers reflect the average number of shoots formed per g of embryogenic callus after 6 weeks on regeneration media.

Carbon Source Maintenance	Carbon Source Regeneration	Shoots per g Fresh Weight	P < 0.01[a]
Suc	Suc	38	a
Suc	Sorbitol	34	a
Sorbitol	Suc	119	b
Sorbitol	Sorbitol	209	c

[a] Letters signify statistically similar (same letter) or different (different letter) growth rates by orthogonal contrasts (P < 0.01), comparing maintenance carbon sources × regeneration carbon source across all weeks.

Table V. *NAD-dependent SDH activity in various tissues of developing maize kernels, seedling tissues, and callus tissues*

Approximately 100 kernels were harvested 21 dpp from field-grown plants and dissected to produce kernel bases and embryos. Endosperms dissected from 10 kernels were cut into thirds (basal, middle, and apical) and respective sections from each kernel were pooled. Leaf and root tissue was prepared from 15-d-old seedlings germinated aseptically on moist filter paper. Embryogenic callus was from cultures maintained on sorbitol for five subcultures. Nonembryogenic callus was derived from callus growing on Suc.

Tissue	Specific Activity
	$\mu M\ min^{-1}\ mg^{-1}$ protein
Kernel base	41.89
Embryo	18.09
Endosperm (basal)	68.71
Endosperm (middle)	34.95
Endosperm (apical)	19.58
Pericarp	4.15
Seedling leaf	N.D.[a]
Seedling root	N.D.
Embryogenic callus	24.62
Nonembryogenic callus	0.21

[a] N.D., No activity detected within the limits of detection of the assay.

nonembryogenic callus also had virtually no activity. The nonembryogenic callus used for these experiments was derived from callus on media containing Suc since none could be obtained from callus grown on sorbitol. SDH activity for embryogenic callus was determined for callus grown on Suc and for callus grown on sorbitol. The specific activity of SDH in embryogenic callus did not differ appreciably whether the callus was grown on media containing Suc or sorbitol (data not shown). The data for embryogenic callus in Table V are from callus grown on sorbitol.

Observations on Inbred Lines and Hybrids Other than H99

Observations of the growth and regeneration of H99 callus presented above have also been noted in several other inbred lines including Mo17, B73, A634, Il451b, and in *Zea diploperennis*. Although specific growth rates and culture morphologies vary from one line to another, the general trends for each of these were observed to be similar to those observed for H99. Specifically, all lines tested grew on sorbitol and formed only embryogenic callus on sorbitol, and the number of plants regenerated per g of callus tissue was greater for sorbitol-grown callus than for Suc-grown callus. In our hands, H99 is an average performer, especially with respect to callus initiation, and appears to be representative of the type of growth to be expected in many other lines.

DISCUSSION

The observations presented here indicate that sorbitol supports the growth of embryogenic callus of maize but not nonembryogenic callus, whereas Suc supports the growth of both types of callus. Overall growth rates are lower on sorbitol than on Suc. However, the principal reason for this difference in growth rate appears to be directly related to the fact that nonregenerable, nonembryogenic callus does not grow on sorbitol but does grow on Suc. It appears that when embryogenic callus is considered alone, growth rates are similar on Suc and sorbitol. The nonembryogenic callus that forms when cultures are maintained on Suc must be removed periodically to sustain the growth of the embryogenic callus. Thus, culture maintenance is simplified substantially by growing cultures on sorbitol, since selection for embryogenic callus is accomplished simply by growing cultures on sorbitol. Ambiguous and tedious visual selection is thereby eliminated.

To our knowledge this is the first report that cereal cultures can be grown on sorbitol as a carbon source. Although sorbitol used in conjunction with Suc had beneficial effects on morphogenesis of rice (Kishor and Reddy, 1986), wheat, and barley cultures (Ryschka et al., 1991), there is no evidence that sorbitol is utilized as a carbon source by these systems. In fact, rice cultures would not sustain growth on sorbitol alone (Kishor and Reddy, 1986). Rather, sorbitol was suggested to serve as an osmoticum that imparted beneficial effects on cereal embryogenic systems. By comparison, the present work showed that sorbitol had beneficial effects on maize cultures at concentrations where little osmotic effect would be apparent, and supported sustained growth of embryogenic callus in the absence of other carbon sources.

The number of shoots regenerated from a given amount of callus was substantially greater if callus was grown on sorbitol rather than on Suc. The differences in regeneration capacity were not due to differences in amounts of embryogenic callus, since only embryogenic callus was transferred to regeneration media. Rather, it would appear that embryogenic callus grown on sorbitol had a greater regenerative capacity than similar embryogenic callus grown on Suc. Some of this difference could be accounted for by differences in the size of embryoids formed. Embryoids formed on sorbitol tended to be smaller than those formed on Suc. Thus, more sorbitol-grown embryoids could be packed into a given volume (weight) of callus than if the embryoids had developed on Suc. However, this would not appear to fully account for the substantial differences in regenerative capacity that have been observed. It is plausible that the balance of metabolic processes is suboptimal for embryogenesis and regeneration when callus is grown on Suc and that a more nearly optimal situation arises when cultures are maintained on sorbitol.

The fact that only embryogenic callus grew on sorbitol and nonembryogenic callus grew only on other carbon sources including Suc suggested the hypothesis that embryogenic callus of maize contains the enzymology necessary to metabolize sorbitol and that nonembryogenic callus does not. SDH was high in embryogenic callus and the level of this activity was similar to the level of activity observed in developing kernel tissues including endosperm and embryo. The level of activity in nonembryogenic callus was very low or virtually nonexistent. Thus, sorbitol metabolism did appear to differ between embryogenic and nonembryogenic callus, and SDH was an enzyme that metabolically distinguished embryogenic callus from nonembryogenic callus.

In the Rosaceae and Plantaginaceae, sorbitol and Suc are both common as primary translocated sugars (Briens and Lahrer, 1983; Loescher, 1987) and sorbitol was shown to

support callus growth and the growth of shoot tip cultures in several of the members of the Rosaceae and Plantaginaceae (Coffin et al., 1976; Negm and Loescher, 1979; R.D. Locy, unpublished data). NAD-dependent SDH, NADP-dependent SDH (aldose reductase), sorbitol-6-P dehydrogenase, and sorbitol oxidase have all been identified in various apple tissues (Negm and Loescher, 1979; Loescher et al., 1982; Beruter, 1985; Yamaki and Ishikawa, 1986). The activity of these enzymes varied from tissue to tissue and from season to season but seemed to be fairly ubiquitous within the whole plant (Yamaki and Ishikawa, 1986). Suc is thought to be the primary translocated sugar in maize, and it was found that sorbitol was not translocated at all and was found only in the developing kernel (Shaw and Dickinson, 1984). An NAD-dependent SDH was detected in maize kernels, but other enzymes of sorbitol metabolism have not been identified in maize (Doehlert, 1987; Doehlert et al., 1988; R.D. Locy, unpublished data). Unlike the enzymes of sorbitol metabolism in apples, in maize these enzymes appear to be restricted to very specific tissues, namely endosperm and embryos.

The developmental profile associated with the presence of sorbitol and related SDH activity in maize kernels and the absence of sorbitol and SDH in other tissues of maize suggest that sorbitol and sorbitol metabolism may be important specifically for embryo development in vivo. Sorbitol began to accumulate in maize kernels between 7 and 14 dpp and starch began to accumulate shortly thereafter (Shaw and Dickinson, 1984). Coincidentally, Lu et al. (1983) showed that the most responsive embryos in terms of embryogenic callus formation were those in which starch was just beginning to accumulate in the scutellum. Here we found SDH activity specifically in embryogenic callus, and it was very low or absent in nonembryogenic callus. It appears that the developmental "window" during which embryogenic callus is obtained from cultured, immature embryos of maize coincides with the "window" during which SDH is active in maize kernels. The link between embryogenesis and sorbitol metabolism is not yet completely clear, but there does appear to be a link between the two.

Bartels et al. (1991) have shown that a gene encoding an aldose reductase that had the capacity to convert Glc into sorbitol was induced in developing barley embryos at about the time when the embryos became desiccation tolerant. These authors argue that sorbitol may play an osmoregulatory role in the developing barley embryo as the seed begins to dehydrate during maturation. The data presented here on sorbitol utilization and SDH activity in maize embryogenic callus and kernel tissues, along with the reports of osmotic effects of sorbitol on rice, wheat, and barley embryogenic callus discussed above (Kishor and Reddy, 1986; Ryschka et al., 1991), further corroborate the idea that sorbitol may have such an osmoregulatory role in embryos of monocotyledonous species. The maize embryogenic callus system may provide a readily manipulatable model system for further investigation of this relationship.

A number of carbohydrates besides Suc are translocated in plants (for review, see Loescher, 1987). These carbohydrates vary from genus to genus and even from species to species to the extent that they can be useful for taxonomic classification of individuals. It is also well documented that tissues of a plant may contain and utilize different carbohydrates than other tissues in the same plant. It is not surprising then that carbon sources other than Suc might be effective in promoting optimum growth responses of a given species or even of a given tissue type in vitro. The effects of only a few of these carbohydrates on culture growth rates and morphology have been examined to a very limited extent. It is apparent that in maize, sorbitol plays a critical role in the development of the embryo and can be utilized to procure a desired effect in maize culture morphogenesis.

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